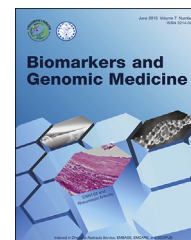
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REVIEW

Use of circulating microRNAs as biomarkers in critically ill polytrauma patients



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Abstract MicroRNAs species are short, noncoding RNAs formed of 19–24 nucleotides. MicroRNAs play an essential role in the regulation of the humoral biochemical processes, cellular proliferation, or other biological processes in the human body. Tissue injuries, biochemical dysfunction, and physiological imbalances are followed by a significant change in the expression of microRNAs in biological fluids. With the recent advances in bioanalysis techniques, it is now possible to identify the microRNA species in biological fluids in specific situations for polytrauma patients. In this paper, we present the importance and the clinical significance of using microRNAs as diagnostic biomarkers for critically ill polytrauma patients.

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Introduction

MicroRNAs species are short, noncoding RNAs formed of 19–24 nucleotides (nt).¹ MicroRNA was first isolated in *Caenorhabditis elegans* in the early 1990s.² Initially, only intracellular microRNA species were identified; however, with the advancement of research in this area, many microRNA families were isolated in different body fluids. So far, about 700 species of microRNA were identified in *Homo sapiens*.² The characteristic features of these species are their high stability and specificity and selectivity. Recent studies have demonstrated that the diversity in the biological pathways through which microRNAs are formation can serve as an important feature for utilizing them as markers for prognosis of disease.³ In addition, their presence in significant concentrations in plasma or other body fluids makes them ideal as biomarkers in clinical practice.

In this paper, the authors present different ways of using microRNA as a specific biomarker for the multiple trauma patients.

Biochemical characteristics of microRNAs

From the biochemical point of view, microRNAs are short RNA species composed of 19–24 nt. The biogenesis of microRNAs begins in the nucleus with the transcription of protein-encoding genes by RNA polymerase II, which produces a primary form of microRNA (pri-microRNA).⁴

Through the polyadenylation of pri-microRNA, the precursor for the synthesis of mature microRNAs (pre-microRNA) is obtained. For this reaction to occur, RNA polymerase III (Drosha) and the DiGeorge critical region 8 complex are required.⁵ Once the pre-microRNA is synthesized, it is transported to the cytoplasm by the nucleocytoplasmic transporter (Exportin-5).⁶ Once inside the cytoplasm, through the action of Dicer complex, the pre-microRNA forms double-stranded mature microRNA (19–24 nt) and microRNA* (passenger strand), which is subsequently degraded by argonaute. The synthesized microRNA is captured by the RNA-induced silencing complex.^{6,7} The microRNAs reach the systemic circulation through the passage across the cell membrane by two specific mechanisms, namely, passive release and active release. In the case of passive release, microRNAs are released into the plasma during cell death in the form of apoptotic bodies. Active release refers to the release of microRNAs into the plasma in the form of microvesicles, exosomes, and high-density lipoproteins particles (Figure 1).^{8,9}

Bioanalytical aspects

In the human body, circulating microRNAs can be identified in a number of fluids such as venous blood, serum, plasma, saliva, urine, cerebrospinal fluid, seminal fluid, pleural fluid, peritoneal fluid, amniotic fluid, tears, and bronchial lavage (Table 1).^{10,11} Weber et al¹⁰ have identified the

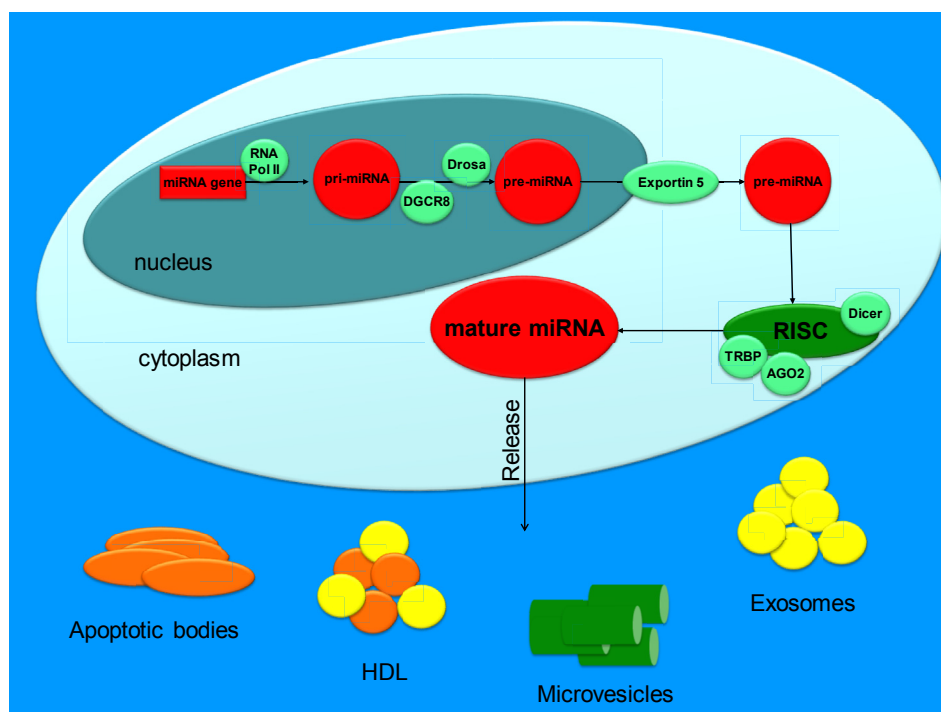


Figure 1 The microRNA biogenesis involves multiple steps requiring RNA polymerase (Pol) II for transcription of the 1–4-kb primary transcript called “pri-microRNA,” nuclease Drosha-DGCR8 for cropping of the single-stranded sequences flanking double-stranded stem-loop structure of the pre-microRNA precursor of the 70-nt long, export of the pre-microRNA via Exportin-5 from the nucleus to the cytoplasm, and nuclease Dicer cleavage of the loop to generate the mature 22-nt long microRNA that will be incorporated into the RNA-induced silencing complex (RISC). ago2 = argonaute; DGCR8 = DiGeorge critical region 8; HDL = high-density lipoprotein; TRBP = trans-activator RNA binding protein.

Table 1 Specificity of microRNAs in biological fluids of the human organism.

Body fluid	microRNAs	References
Plasma	microRNA-224, microRNA-483-3p, microRNA-518f, microRNA-508-3p, microRNA-551b, microRNA-182, microRNA-135a, microRNA-139-3p, microRNA-801, microRNA-369-3p, microRNA-519d, microRNA-299-5p, microRNA-373, and microRNA-330-5p	10,15,16
Venous blood	microRNA-16, microRNA-20a, microRNA-106a, microRNA-126, microRNA-150, microRNA-185, microRNA-451, and microRNA-451a	13
Tears	microRNA-637	4
Saliva	microRNA-182, microRNA-450b-5p, microRNA-622, microRNA-141, microRNA-26a, microRNA-145, microRNA-135b, microRNA-281, microRNA-96, microRNA-1228, and microRNA-431	10,15
Semen	microRNA-508-5p, microRNA-644, microRNA-17, microRNA-380, microRNA-29b-2, and microRNA-240	13
Amniotic fluid	microRNA-636, 92a-1, microRNA-26b, microRNA-376b, microRNA-556-5p, and microRNA-593	13,15
Peritoneal fluid	microRNA-129, microRNA-583, microRNA-223, microRNA-627, and microRNA-29b-1	15
Colostrum	microRNA-18a, microRNA-513a-50, microRNA-10b, microRNA-192, microRNA-193b, and microRNA-130a	10
Menstrual blood	microRNA-185, microRNA-144, microRNA-412, and microRNA-451	10,13
Vaginal secretion	microRNA-617 and microRNA-891a	10,16,17
Breast milk	microRNA-10a, microRNA-28-5p, microRNA-150, microRNA-193b, microRNA-217, microRNA-518c, and microRNA-92b	15
Cerebrospinal fluid	microRNA-577	15

concentration of microRNAs in 12 biological fluids.¹⁰ Based on their study results, the existence of a large number of microRNAs in these fluids was reported: 359 microRNA species in amniotic fluid, 429 in breast milk, 260 in bronchial lavage, 212 in cerebrospinal fluid, 386 in colostrum, 397 in peritoneal fluid, 359 in plasma, 458 in saliva, 436 in seminal fluid, 320 in tears, and 204 in urine.^{11,12} Zubakov et al¹³ evaluated the expression of microRNAs in five types of biological fluids by microarray and reverse transcription-polymerase chain reaction data analyses, and reported the existence of a series of microRNAs, including the following: microRNA-185, microRNA-144 (menstrual blood), microRNA-20a, microRNA-106a, microRNA-185 (venous blood), microRNA-943, microRNA-135a, microRNA-10a, microRNA-507 (semen), microRNA-583, microRNA-518c,

microRNA-208b (saliva), microRNA-617, and microRNA-891a (vaginal secretion). To be used as biomarkers, microRNA species must fulfill a number of features, among which the most important are low cost, simple techniques for isolation and analysis, noninvasive techniques, and high specificity and selectivity. Analytical methods for identifying microRNAs present in these fluids are hybridization (microarray), quantitative reverse transcription-polymerase chain reaction, and RNA sequencing.^{13,14}

Circulating microRNAs in cancer

A series of studies suggested the possible link between the expression of microRNAs and different forms of cancer. The

Table 2 Specific microRNA species for the oncologic diagnosis.

Oncological pathology	microRNAs	References
Breast cancer	microRNA-10b, microRNA-341, and microRNA-195	15,22,23
Prostate cancer	microRNA-16, microRNA-92a, microRNA-103, microRNA-107, microRNA-197, microRNA-34b, microRNA-328, microRNA-485-3p, microRNA-92b, microRNA-636, microRNA-640, microRNA-766, and microRNA-885-5p	15,20,24,25
Ovarian cancer	microRNA-21, microRNA-92, microRNA-93, microRNA-126, and microRNA-92a	15,26
Lung cancer	microRNA-7i, microRNA-146b, microRNA-206, microRNA-21, microRNA-10b, microRNA-15b, microRNA-19b, microRNA-182, microRNA-197, microRNA-222, microRNA-320, microRNA-375, microRNA-574-5p, microRNA-660, and microRNA-1254	15,18,19
Colorectal cancer	microRNA-29a, microRNA-92a, microRNA-378, microRNA-21, and microRNA-18a	27,28
Bladder cancer	microRNA-126 and microRNA-182	28,29
Esophageal cancer	microRNA-1246, microRNA-31, and microRNA-1322	28,30,31
Nasopharyngeal cancer	microRNA-17, microRNA-20a, microRNA-29c, and microRNA-223	1
Renal cancer	microRNA-1233	20
Head and neck carcinoma	microRNA-425-5p and microRNA-93-5p	21
Hepatocellular carcinoma	microRNA-9-3p, microRNA-9, microRNA-10a, microRNA-10b, microRNA-15a, microRNA-18a, microRNA-21, microRNA-27a, microRNA-92, and microRNA-96	11,15,28

most commonly studied types of cancers in these studies are prostate cancer, renal cancer, breast cancer, ovarian cancer, lung cancer, bladder cancer, hepatocellular carcinoma, gastrointestinal cancer, and head and neck cancer.^{15–17} Billeter et al¹⁸ reported an increased plasmatic concentration of the following series of microRNAs in lung cancer: microRNA-10b, microRNA-15b, microRNA-17, microRNA-19b, microRNA-182, microRNA-182, microRNA-197, microRNA-222, microRNA-320, microRNA-375, microRNA-574-5p, microRNA-660, and microRNA-1254. In hepatocellular carcinoma cases, Borel et al¹¹ identified the following series of microRNAs whose plasmatic concentrations are significantly elevated: microRNA-9-3p, microRNA-9, microRNA-10a, microRNA-10b, microRNA-15a, microRNA-18a, microRNA-21, microRNA-27a, microRNA-93, and microRNA-96.¹¹ Wang et al¹⁹ studied the expression of microRNAs in gastrointestinal tumors and reported the increased plasmatic levels for microRNA-601, microRNA-760, microRNA-29a, microRNA-92a. In colorectal cancer cases, microRNA-378, microRNA-21, microRNA-18a have been identified; in esophageal cancer, microRNA-1246, microRNA-31, and microRNA-1322 were identified.²⁰ In renal cancer, Wulfken and collaborators²⁰ highlighted the presence of microRNA-1233. Summerer et al²¹ reported high levels of microRNA-425-5p and microRNA93-5p in head and neck cancer cases. Table 2 presents the microRNA species specific for the oncological pathologies. Based on circulating microRNAs, many previously published studies have highlighted the characteristic features for diagnosis of cancer using noninvasive diagnostic methods. A number of factors support the use of microRNAs as biomarkers for diagnosis including high stability, specificity, and selectivity.^{22–29}

microRNAs as biomarkers for critically ill polytrauma patients

Patients with multiple trauma in the intensive care unit (ICU) present a significant challenge for doctors because of complexity of their conditions (multiple physiological, biochemical, and systemic imbalances as well as the multiple organ damage). The most specific injuries suffered by polytrauma patients that cause a significant decrease in survival rates are brain injuries, spinal cord injuries, severe pulmonary injuries, severe abdominal injuries, and severe burns. In a high percentage trauma patients, severe hemorrhagic shock associated with vascular and cardiac failures has been noted.^{30–34} Post-traumatic secondary complications commonly noted in critically ill trauma patients are microvascular disease, systemic inflammations, sepsis, and multiple organ failure. Physiological, biochemical, and metabolic imbalances are associated with primary injuries. The secondary post-traumatic injuries significantly contribute to the decrease of the survival rate in these patients. Numerous studies have been conducted regarding the identification of expression of microRNAs in the different traumas that play a significant role in reducing the survival rates of critically ill polytrauma patients.

Shortly after a traumatic event, these patients suffer systemic inflammatory response syndrome (SIRS). However, due to excessive production of proinflammatory molecules,

activation of the coagulation cascade, hypermetabolism, and physiological imbalances, SIRS progresses into sepsis.^{35–39} The intensity of the trauma, post-traumatic secondary lesions, and genetic characteristics determine the evolution of SIRS. The following stages are noted after a traumatic event: hyperinflammation (first phase), followed by an equilibrium state, a mixed antagonist response syndrome, which characterized by a decrease in the concentration of proinflammatory mediators and an increase in the concentration of anti-inflammatory mediators. Following this inflammatory stage is the compensatory anti-inflammatory response syndrome (CARS), which is characterized by an increase in the concentration of inflammatory mediators. Depending on a number of pathophysiological aspects, critically ill polytrauma patients may progress to multiple organ dysfunction syndrome (MODS) from SIRS/CARS (Figure 2). Endothelial dysfunctions are also largely responsible for augmenting the inflammatory response. The activation of nuclear factor transcription-kB (NF-kB) modulates the activation of specific adhesion molecules, such as vascular cell adhesion molecules-1, intercellular adhesion molecules-1, and E selectin. Moreover, the activity of NF-kB is responsible for the modulation of some proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukin-1-beta (IL-1 β). Sun et al⁴⁰ studied the role of microRNAs in the NF-kB pathway and reported that microRNA-181b is responsible for modulating NF-kB pathway in inflammation and sepsis. In addition, it was suggested that in patients with sepsis admitted to ICUs, the expression of microRNA-181b is greatly reduced. Statistically significant correlations between increased TNF- α and IL-1 levels and an increased expression of miRNA-146 have also been reported.⁴¹ Recent studies have shown that a number of microRNAs species are involved in modulating the activity of antigen-presenting cells or T cells. Among these are 17–92 microRNA, microRNA-181, microRNA-146a, microRNA-155, microRNA-511, microRNA-132, microRNA-122, microRNA-21, microRNA-125b, microRNA-187, and microRNA-223.⁴²

Traumatic brain injury (TBI) is responsible for a high percentage of death or disability in surviving patients. Recently, many studies were also carried out on the diagnosis and prognosis of TBI. Assessing the stage of brain trauma based on expression of microRNAs has also been reported.³³ Liu et al³⁴ have analyzed the expression of microRNAs in mitochondrial fractions of rat hippocampal tissue and reported significant increases in the expressions of microRNA-155 and microRNA-223 following TBI. The authors of that study also reported increased plasmatic concentrations of microRNA-142-3p and microRNA-221 after TBI in laboratory animals.³⁴

Sharma et al³⁵ reported changes in some microRNAs species in laboratory animals with TBI. These species include microRNA-199a-3p, microRNA-214-3p, microRNA-218-5p, microRNA-196c, microRNA-31-5p, and microRNA-106b. Liu et al³⁶ carried out a study on the expression of microRNAs in TBI and highlighted significant increases in the expression of microRNA-144, microRNA-153, and microRNA-340-5p. Another important cause of death in the ICU is spinal cord injury (SCI).³⁷ SCI induces a series of secondary injuries caused by the various related physiopathologies that have a direct impact on increased mortality. Godwin et

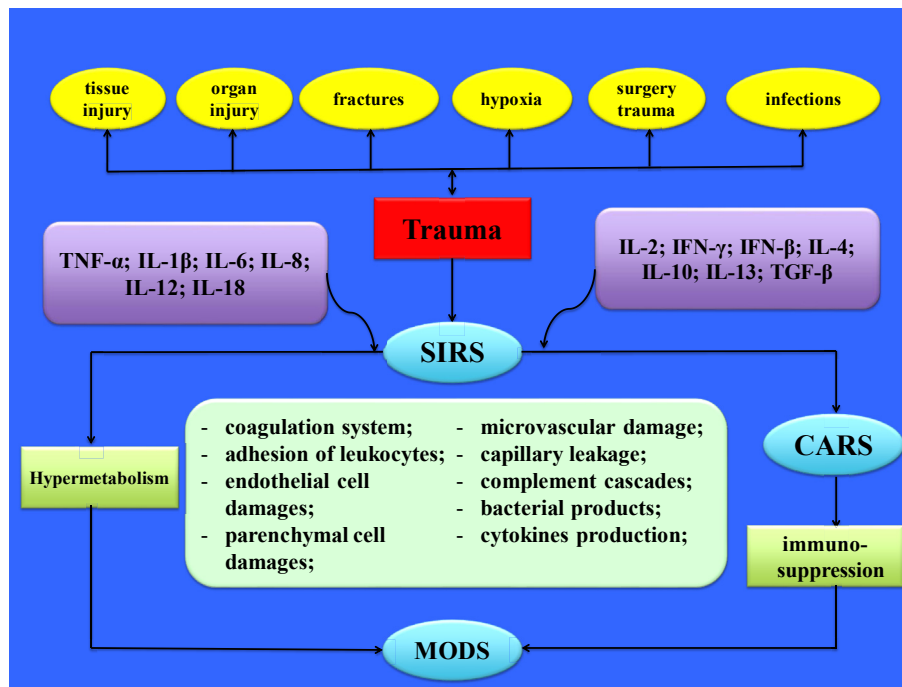


Figure 2 Scheme of inflammation after trauma. IFN = interferon; IL = interleukin; TGF = tumor growth factor; TNF = tumor necrosis factor.

al³⁸ studied the expression of microRNAs in laboratory animals with SCI and reported a decrease in the plasma concentrations of microRNA-137, microRNA-181, microRNA-219-2-3p, and microRNA-7, and an increase in the concentration of microRNA-21.³⁸ A common pathology in polytrauma patients is respiratory failure, which is caused by either direct injuries (rib fractures, hemothorax, pneumothorax, and pulmonary parenchymal injury) or indirect injuries associated with mechanical ventilation or sepsis. Among them, acute respiratory distress syndrome (ARDS) has the most significant impact on worsening clinical status.³⁹ ARDS is mainly characterized by pulmonary injuries and severe inflammation of the lung parenchyma. Huang et al³⁷ conducted an analysis of microRNA expression in laboratory animals with ARDS, and reported significant changes in the expression of 27 microRNA species.³⁷ They reported significant increases in the plasma concentrations of microRNA-344, microRNA-346, microRNA-99a, microRNA-127, microRNA-128b, microRNA-135b, microRNA-30a, and microRNA-30b, but decreases in the plasma concentrations of microRNA-24, microRNA-26a, and microRNA-126.³⁷ Kidney damage is another significant concern while treating critically ill patients. Godwin et al³⁸ reported modifications in the expression of a series of microRNA species after severe renal dysfunction in laboratory animals. Their studies showed a significant increase in the expression of microRNA-21, especially in the case of renal ischemic reperfusion injury. There are numerous studies on the use of circulating microRNAs as biomarkers for the diagnosis of trauma; however, at present, many studies are only conducted on laboratory animals, as no concrete results are available for human patients.

Expression of microRNAs in sepsis

Sepsis is characterized by severe systemic inflammation during generalized infection.^{41,42} In patients admitted to the ICU, sepsis is a leading cause of death because of multiple organ failure. For a polytrauma patient, SIRS is initially triggered, followed by sepsis, severe sepsis, septic shock, and eventually MODS.⁴³ According to the American College of Chest Physicians and the Society of Critical Care Medicine, there are a number of criteria for defining the aforementioned conditions: SIRS is defined by core body temperature greater than 38°C or less than 36°C; heart rate of 90 bpm or greater or more than 20 breaths per minute or PaCO₂ less than 32 mmHg; white blood cell levels of 12,000/μL or greater or 4,000/μL or less, or more than 10% of immature forms; sepsis is defined by at least two SIRS criteria caused by known or suspected infection; severe sepsis is defined by sepsis associated with organ dysfunction or hypotension and hypoperfusion; septic shock is defined by sepsis with persistent or refractory hypotension or tissue hypoperfusion despite adequate fluid resuscitation; MODS is defined by presence of organ dysfunction in an acutely ill patient such that homeostasis cannot be maintained without intervention.⁴⁴ In recent years, research studies in this field have focused on identifying specific biomarkers that can be correlated with the stage and severity of sepsis. The biomarkers used in this sense are C-reactive protein,⁴⁵ procalcitonin,⁴⁵ and IL-6.⁴⁶ Recently, questions have arisen about the use of microRNAs as biomarkers for the prediction and monitoring of sepsis in critically ill patients. A significant number of studies analyzed microRNA profiles in patients with sepsis (Table 3).⁴⁷ At present, a number of

Table 3 Expression of microRNAs species in patients with sepsis.

References	Expression in sepsis patients	References
microRNA-15a, microRNA-16	Up	47
microRNA-122, microRNA-146a, microRNA-223, microRNA-483-5p, microRNA-499-5p, microRNA-574-5p, microRNA-150, and microRNA-193b	Down	47
microRNA-182, microRNA-199a-5p, microRNA-203, microRNA-211, microRNA-222, and microRNA-29b	Up	49
microRNA-233 and microRNA-146	Down	50
microRNA-150 and microRNA-342-5p	Down	46
microRNA-466l	Down	45

microRNAs can be correlated with sepsis. Vasilescu et al.⁴⁸ conducted a study on microRNA species in patients with sepsis, and reported a significant decrease in the circulating microRNA-150 level. In their study, they correlated the decrease in the plasma microRNA-150 level with increased proinflammatory cytokines and IL-18.⁴⁹ Wang et al.⁵⁰ also reported similar significant decreases in the levels of microRNA-223 and microRNA-146. Li et al.⁴⁵ reported decreased plasma levels of microRNA-466l in patients with sepsis. The innate immune response represents the first line of defence in the fight against invading pathogens.⁵¹ Petrocca et al.⁵¹ have analyzed the expression of microRNAs in circulating leukocytes in an *in vivo* experimental study of acute inflammation caused by *Escherichia coli*.⁵¹ The results of their study demonstrated substantial changes in the expression of microRNAs based on a number of factors, including innate pathogen (microRNA-146b, microRNA-150, and microRNA-143); apoptosis (microRNA-150 and microRNA-143); and cytokines (microRNA-342 and microRNA-143).⁵² Inflammatory mediators determine the type of inflammatory response, acting at the systemic level, that has a synergistic or antagonistic effect on other structures and biochemical functions causing adverse effects, including severe tissue injury and death. Inflammatory cascade is triggered by the presence of specific microorganisms and toxins biosynthesized by them.^{47,49–51} As clearly explained in the literature, a number of bacteria produce a single toxin responsible for triggering the respective disease, whereas many others produce exotoxins that are directly involved in toxic shock. Some bacteria produce both exotoxins and endotoxins, whereas some only produce endotoxins. The most frequently implicated toxin in septic shock is endotoxin. This is present in the bacterial wall, as it is a lipid–protein–glucide macromolecular complex.⁵² Endotoxemia is noted in critically ill patients with septic shock, infected by Gram-negative microorganisms, and multiple organ dysfunction. Specific immune response acts in close cooperation with the nonspecific one in phagocytosis and in the processing of infectious antigens.⁵³ Biochemical-mediated immunity represents a gold

goal in the specific immune response in infections by killing the bacteria, neutralizing of toxins and by increasing the phagocytosis. In the current moment for the detection of causative pathogens in sepsis diagnostics is used as an extremely high preponderance the blood cultures.⁵³ A promising method in this regard is the use of specific microRNAs for the detection of microbiological species. Numerous studies have highlighted the role of microRNAs in the pathogenesis of viruses and bacteria. The activity of bacteria in the human body significantly alters the expression of microRNAs, and these changes are considerably noticeable. Moreover, the expression of microRNAs is modified according to different immune responses in bacterial infections. A number of studies have demonstrated an increase in the expression of microRNA-146 and microRNA-155 in *Helicobacter pylori*, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Salmonella enterica* infections.^{53–58} Zheng et al.⁵⁶ studied the expression of microRNAs in the case of *Brucella melitensis* and reported the presence of microRNA-92a, microRNA-93, microRNA-181b, and microRNA-1981. In *Pseudomonas aeruginosa* infections, significant changes in the expression of microRNA-302b and microRNA-233 were reported. Hsieh et al.⁵⁹ studied the expressions of microRNAs species in laboratory mice injected with lipoteichoic acid specific for Gram-positive bacteria and those injected with lipopolysaccharide specific for Gram-negative bacteria. Their study results showed significant increases in the levels of microRNA-451, microRNA-668, microRNA-1902, and microRNA-1904.⁵⁹ Thus, their study results provided a new perspective to differentiate between Gram-positive and Gram-negative bacterial infections.

Conclusion

Because of their characteristics and functions, microRNAs are being increasingly studied and used as a biomarker for a number of diseases in the clinical field. MicroRNAs released by a tissue during tissue aggression or a period of prolonged stress (inflammation, infection, sepsis, or organ dysfunction) make them an ideal candidate for use as a diagnostic biomarker in the bioanalytical procedures of diagnosis. In addition, microRNAs can also be used to carry out specific treatments to modulate at the molecular level a number of specific physiological pathways for the critically ill patients. In conclusion, we can affirm that improving and strengthening diagnostic methods based on identifying microRNAs are impetuous required.

Conflicts of interest

The authors have no conflicts of interest to declare.

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